

Sink Metabolism in Tomato Fruit¹

II. PHLOEM UNLOADING AND SUGAR UPTAKE

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ABSTRACT

Analysis of [³H]-fructosyl-sucrose translocation in tomato (*Lycopersicon esculentum* Mill.) indicates that phloem unloading in the fruit occurs, at least in part, to the apoplast followed by extracellular hydrolysis. Apoplastic sucrose, glucose, and fructose concentrations were estimated as 1 to 7, 12 to 49, and 8 to 63 millimolar, respectively in the tomato fruit pericarp tissue. Hexose concentrations were at least four-fold greater than sucrose at all developmental stages. Short-term uptake of [¹⁴C]sucrose, -glucose, and -fructose in tomato pericarp disks showed first order kinetics over the physiologically relevant concentration range. The uptake rate of [¹⁴C]-(glucosyl)-1'-fluorosucrose was identical to the rate of [¹⁴C]sucrose uptake, suggesting sucrose may be taken up directly without prior extracellular hydrolysis. Short-term uptake of all three sugars was insensitive to 10 micromolar carbonyl cyanide *m*-chlorophenylhydrazine and to 10 micromolar *p*-chloromercuribenzenesulfonic acid. However, long-term accumulation of glucose was sensitive to carbonyl cyanide *m*-chlorophenylhydrazine. Together these results suggest that although sucrose is at least partially hydrolyzed in the apoplast, sucrose may enter the metabolic carbohydrate pool directly. In addition, sugar uptake across the plasma membrane does not appear to be energy dependent, suggesting that sugar accumulation in the tomato fruit is driven by subsequent intracellular metabolism and/or active uptake at the tonoplast.

photosynthate appears to be controlled by the metabolic activity of the fruit (22, 23) and inversely related to fruit sucrose levels (24). Although sucrose is the primary form of translocated sugar in the tomato (22), the two hexoses, glucose and fructose, in approximately equal amounts, make up approximately 53% of the total soluble solids of the fruit, while the sucrose contribution is very small (4). The pathway and mechanism of phloem unloading into the fruit is not well characterized, but there is evidence for the existence of a cell wall-bound invertase in the pericarp (13, 16). The existence of this extracellular invertase suggests that sucrose unloading in the tomato follows the same path as that in corn kernels (9) and sugarcane stalk parenchyma (7). In these systems, sucrose is unloaded in the apoplast where it is hydrolyzed by invertase forming glucose and fructose. The resultant hexoses are then taken up by the sink cells.

Tomato fruit pericarp tissue was used in this study because the pericarp has been shown to contain 20% more reducing sugars than the locule (20). Three genotypes that differ significantly in their soluble solids content were studied cv LA1563, cv VF145-7879, and cv UC82B, with soluble solids values of 6.3, 5.5, and 4.6 °Brix, respectively. LA1563 is a high soluble solids breeding line derived via a backcross program from a cross between *Lycopersicon esculentum* cv 204B and *L. chmielewskii* accession LA1028, a wild, green-fruited species that accumulates sucrose and has a soluble solids value of 10 °Brix (25). VF145-7879 and UC82B are commercial processing tomato varieties.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Three processing tomato (*Lycopersicon esculentum* Mill.) genotypes, cv VF145-7879 (7879), cv UC82B (UC82B), and cv LA1563 (1563) were sown in seedling trays and transplanted to the field in June or to a bed greenhouse 6 weeks later. Field-grown plants received standard cultural practices for processing tomatoes in California. Bed greenhouse-grown plants received only natural lighting with a 24°C day/18°C temperature regime and were fertilized twice weekly (Plantex 15:15:15). Fruit age as DAA,⁴ was determined by tagging the truss when the two proximal flowers were open. All other floral buds were removed to ensure uniform development of the remaining two (1).

Determination of Apoplastic Volumes. The apoplastic volume of fruit pericarp disks was determined in all three tomato genotypes using compartmental analysis of efflux kinetics (12). Throughout this paper, the apoplast is defined as the intercellular space and cell wall, while symplast refers to the cytoplasm and vacuole of the tomato pericarp cells. Three fruits from each genotype at 15, 20, 30, 40, and 50 DAA were picked and three

Tomato fruit quality is largely determined by the content of soluble solids, with soluble sugars being the major component of the soluble solids fraction. Genetic improvement of tomato soluble solids content has often been associated with a decrease in yield (21), although the extremely high soluble solids content of certain wild species (18) suggests that the genetic and physiological potential exists for achieving higher soluble solids levels in commercial tomato varieties. An understanding of the transport and metabolic pathways associated with soluble sugar accumulation in tomato fruit may lead to novel selection criteria or molecular genetic strategies to improve tomato fruit soluble sugar levels.

Little is known about the mechanisms underlying sugar accumulation in the developing tomato fruit. Several studies have indicated that the sink appears to be limiting under normal growing conditions in the tomato (14, 15). The rate of import of

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⁴ Abbreviations: DAA, Days after anthesis; 1FS, (glucosyl)-1'-fluorosucrose; CCCP, carbonyl cyanide *m*-chlorophenylhydrazine; PCMBS, *p*-chloromercuribenzenesulfonic acid; 3-O-MG, 3-O-methylglucose; FW, fresh weight.

1 cm diameter discs removed from the equator of the fruit using a 1 cm cork borer. The cuticles were removed and the discs were incubated for 1 h in an aerated ^3H -inulin solution (Amersham Corporation, 1.5 $\mu\text{Ci}/\text{nmol}$) at 2°C . The discs were stirred for 15 s in 1 L of water to remove surface radioactivity and then were consecutively washed in 2 ml volumes of water over a 6 h period at 2°C . The amount of radioactivity in each 2 ml wash was determined by liquid scintillation spectrometry. The discs were oven-dried and oxidized (Packard Tri-Carb B306 sample oxidizer) to determine the amount of radioactivity remaining in the tissue.

Determination of Apoplastic Sugar. From the efflux kinetics of the ^3H -inulin an apoplastic efflux time of 6 to 8 min was determined. Three pericarp discs from three fruit at each developmental stage were stirred for 15 s in 1 L of cold water to remove sugars from cut cells and then were placed in 10 ml of aerated cold water for 8 min to efflux the apoplastic sugars. These washes were then analyzed on an HPLC system (Beckman model 110A pump, Altex injector system, BioRad HPX-87C carbohydrate analysis column and a Altex 156 refractive index detector), and the amounts of sucrose, glucose, and fructose were determined.

Labeling with [^3H]-Fructosyl-Sucrose. Six tomato plants (cv 7879) grown in 2 gallon pots in the greenhouse were pruned to a single leaf and subtending 20 DAA fruit 24 h prior to the experiment. A single leaflet on each plant was abraded with carborundum between two lateral veins and the abraded area was enclosed in a silicon well. Thirty μCi of [^3H]-fructosyl-sucrose (10.1 Ci/nmol) was applied to each well in 100 μl of a low salt buffer (0.1 mM MgSO_4 , 0.5 mM KCl, 0.5 mM CaCl_2 , and 5 mM Mes, pH 6.0), and the well was covered with a cover slip. After 2 and 6 h, discs were harvested from around the equator of the fruit and washed for 8 min to efflux the apoplastic sugars. The symplastic sugars were extracted by incubating the discs twice in 80% ethanol at 80°C for 30 min. The symplastic and apoplastic fractions were analyzed by HPLC and the amount of radiolabeled sucrose, glucose, and fructose was determined by liquid scintillation spectrometry.

Synthesis of [^{14}C]-1'-Fluorosucrose. 1FS was synthesized from 1'-fluoro-fructose (provided by Dr. W. Hitz) and [^{14}C]UDPglucose according to the procedure of Card and Hitz (3). The specific activity of the [^{14}C]1FS was 275 $\mu\text{Ci}/\mu\text{mol}$.

Invertase Isolation and Assay. Invertase was isolated according to the procedure of Nakagawa *et al.* (17) with modifications. This procedure resulted in a 51-fold purification of tomato cell wall invertase. All protein concentrations were determined by the method of Bradford (2), and invertase assays used either 10 mM [^{14}C]sucrose or [^{14}C]1FS as substrate. Products of the invertase reaction were analyzed by paper chromatography using isopropanol:butanol: H_2O (7:1:2), and the radioactivity associated with hexose was determined by scanning the paper chromatograms with a Packard Radio-Scanner.

In Vitro Sugar Uptake. The mechanism of sugar uptake across the tomato pericarp plasma membrane was examined using pericarp discs harvested from field-grown 20 DAA fruit of the three genotypes. After removing the cuticle, the discs were weighed and preincubated at 25°C in aerated buffer containing 5 mM KCl, 0.5 mM NaCl, 0.1 mM CaCl_2 , 0.1 mM MgCl_2 , and 5 mM Mes (pH 6.0) for 30 min. The discs were then placed in fresh buffer containing either (U- ^{14}C)sucrose (0.2 $\mu\text{Ci}/\mu\text{mol}$, ICN), D(U- ^{14}C)glucose, or D(U- ^{14}C)fructose (each 0.2 $\mu\text{Ci}/\mu\text{mol}$, Amersham Corp.) at concentrations varying from 0.5 to 20 mM for sucrose and 1 to 75 mM for the hexoses. The discs were then rinsed twice in 10 ml of aerated buffer for 4 min each to remove apoplastic sugars. The amount of labeled sugars remaining in the symplast was determined by solubilizing the discs in tissue solubilizer (NCS) at 50°C for 12 h and resuspending these solu-

tions in Liquiscint (National Diagnostics) for liquid scintillation spectrometry.

In a separate experiment, the uptake rates of [^{14}C]1FS (10 μM , 0.2 $\mu\text{Ci}/\mu\text{mol}$) and [^{14}C]sucrose (10 μM , 0.2 $\mu\text{Ci}/\mu\text{mol}$) were compared over a time course from 5 to 45 min. The amount of radiolabel in the symplast was determined as described above.

Effect of Inhibitors on Sugar Uptake. Discs from 20 DAA field-grown 1563 plants were prepared as described above and preincubated in aerated buffer with and without 10 μM PCMBs or 10 μM CCCP. The discs were then incubated in aerated buffer containing 1 mM [^{14}C]glucose, sucrose, or fructose (all 0.2 $\mu\text{Ci}/\mu\text{mol}$) for 30 min in the presence or absence of 10 μM PCMBs or 10 μM CCCP.

In a separate experiment, we examined the effect of 10 μM CCCP on short- and long-term uptake of 1 mM [^{14}C]glucose, a metabolized sugar, and 1 mM [^{14}C]-3-O-MG, an unmetabolized glucose analog that has been shown in other plant systems to be absorbed by plasma membrane glucose carriers. Discs were preincubated for 30 min in the presence or absence of 10 μM CCCP and then incubated in 1 mM [^{14}C]glucose (0.2 $\mu\text{Ci}/\mu\text{mol}$) or 1 mM [^{14}C]-3-O-MG in the presence or absence of 10 μM CCCP over short- (10–30 min) and long-term (1–3 h) incubation periods. The amount of radiolabel in the symplast was determined as described above.

RESULTS AND DISCUSSION

Apoplastic Volume and Sugar Concentration. In order to measure apoplastic volumes and to select appropriate elution times for sampling apoplastic solutes, compartmental analysis of the efflux kinetics of preloaded [^3H]inulin was carried out (Fig. 1). The data in Figure 1 could be analyzed in terms of the efflux of [^3H]inulin from three compartments in series having $t_{1/2}$ for efflux of approximately 2 min, 20 min, and greater than 200 min. Linear regression analysis of each set of efflux data (Fig. 1, inset) allowed the determination of radioactivity in each compartment at time zero. Using an estimate of the apoplastic radioactivity at time zero, the apoplastic volume was calculated as described previously (12). The apoplastic volumes of tomato pericarp tissue from the three genotypes ranged from 17 to 71 $\mu\text{l}/\text{g}$ FW over development. Apoplastic volumes were relatively constant throughout fruit development except at the later stages of maturity when the apoplastic volume increased sharply (Fig. 2).

Based on the compartmental analysis of efflux kinetics, an

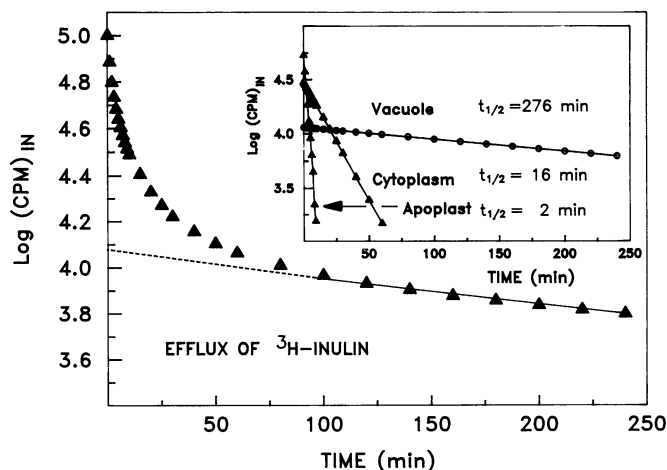


FIG. 1. Efflux kinetics of ^3H -inulin from a tomato fruit pericarp disc. Line indicates efflux from the slowest exchanging compartment. After sequential subtraction of the slower exchanging compartments, efflux from three compartments could be resolved (inset).

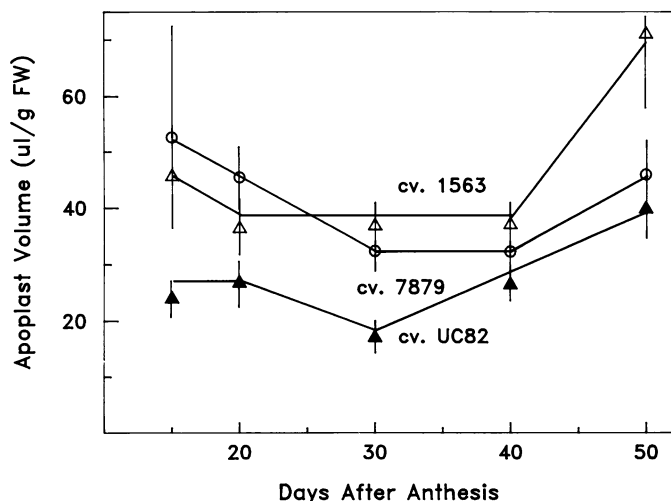


FIG. 2. Apoplast volumes of tomato fruit pericarp tissue. Volumes were determined by compartmental analysis of efflux kinetics of ^3H -inulin in three cultivars, cv 1563 (Δ), cv 7879 (\circ), and cv UC82 (\blacktriangle) at the indicated developmental stage. Each point represents the mean and SE of three replicates.

apoplastic efflux time of 8 min was determined to be appropriate for sampling apoplastic solutes. Soluble sugar levels in the apoplast were determined throughout fruit development in the three tomato varieties (Fig. 3). Glucose and fructose concentrations ranged from 8 to 60 mM in the apoplast and were present in a ratio of approximately 1:1 in all samples. Sucrose concentrations in the apoplast were much lower throughout development than hexose concentrations. These low sucrose concentrations may reflect hydrolysis of sucrose in the apoplast by an extracellular invertase.

Pathway of Phloem Unloading. To ascertain whether the apoplastic sugar composition reflected an apoplastic route of phloem unloading or simply equilibration of the apoplast and symplast, we labeled leaves with [^3H]-(*fructosyl*)-sucrose. The sugars in apoplast and symplast samples of fruit pericarp were analyzed following a 2 or 6 h labeling. Because sucrose is the translocated sugar, the presence of an asymmetrically labeled hexose pool in the apoplast sample could only arise from apoplastic unloading and hydrolysis, since a symplastic route of unloading and hydrolysis would result in the randomization of ^3H -label due to the activity of hexose isomerase (6). In fact, the apoplastic hexose pool was asymmetrically labeled (Table I), and in all cases the degree of asymmetry of labeling of the apoplastic hexose pool was greater than the asymmetry in the symplastic hexose pool. This result suggests that the apoplastic hexose pool did not arise from equilibration of the apoplast and symplast and that sucrose may be unloaded to the apoplast and hydrolyzed. In our apoplast samples, sucrose was not exhaustively hydrolyzed and significant amounts of symplastic sucrose were detected, suggesting that only a portion of the translocated sucrose undergoes extracellular degradation. With longer periods of leaf labeling prior to fruit sampling, symmetric labeling of the apoplastic hexose pool was observed (25), due to greater isomerization of the [^3H]sucrose along the translocation pathway.

Sugar Uptake Kinetics. The uptake of radiolabeled glucose, fructose, and sucrose was measured in excised pericarp discs from all three tomato cultivars. Over a physiological concentration range of glucose and fructose (1–75 mM), uptake kinetics were linear and nonsaturating (Fig. 4). Rates of uptake of the hexoses were also similar in all three tomato cultivars. Over a physiological concentration range for sucrose (0.5–20 mM), uptake was also linear and nonsaturating (Fig. 5). In other experiments, sucrose uptake did not show signs of saturation at 75 mM

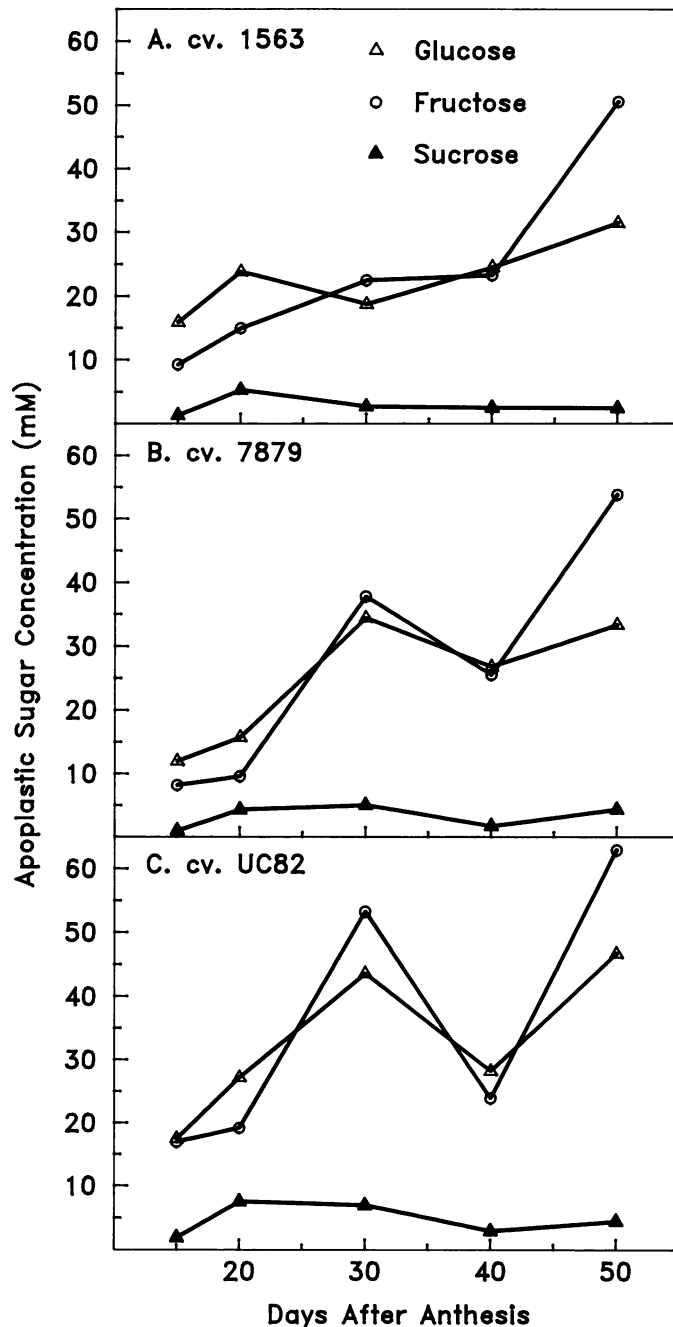


FIG. 3. Apoplast concentration of soluble sugars in cv 1563 (A), cv 7879 (B), and cv UC82 (C), at the indicated developmental stage. Sugar content of apoplast samples as determined by HPLC (see "Materials and Methods") and apoplast volumes from Figure 2 were used to calculate the apoplast concentration of each sugar. Each point represents the mean of three determinations.

sucrose (data not shown). Interestingly, sucrose uptake was consistently higher in the high soluble solids tomato cultivar, cv 1563 (Fig. 5).

To determine whether sucrose was being taken up directly by tomato pericarp cells or by a mechanism involving extracellular hydrolysis followed by hexose uptake, uptake of an invertase-resistant sucrose analog (IFS) was examined. It has previously been demonstrated that IFS is resistant to yeast invertase but is recognized by sucrose transport carriers (10). We verified that this sucrose analog is also resistant to invertase isolated from tomato fruit (Fig. 6) and found that the uptake rate of IFS was

Table I. Radioactivity in Soluble Sugars in the Apoplast and Symplast of Tomato Fruit Pericarp following Labeling of a Source Leaf with [^3H]-fructosyl)-sucrose for 2 or 6 h

Apoplastic solutes were sampled by an 8 min buffer rinse of pericarp discs, and then symplastic solutes were extracted from the same tissue sample in 80% ethanol. Sugars were separated by HPLC, and radioactivity in glucose, fructose, and sucrose fractions was determined.

Sample No.	Glucose	Fructose	Sucrose	Glu/Fru ^a
	% of total cpm			
1 ^b Apoplast	0	60.0	40.0	0
Symplast	9.7	23.1	67.2	0.42
2 ^c Apoplast	0	32.0	68.0	0
Symplast	8.7	6.8	84.5	1.26
3 ^c Apoplast	0	47.0	53.0	0
Symplast	6.8	24.6	68.5	0.28

^a Ratio of ^3H in the hexoses. ^b 2 h leaf labeling. ^c 6 h leaf labeling.

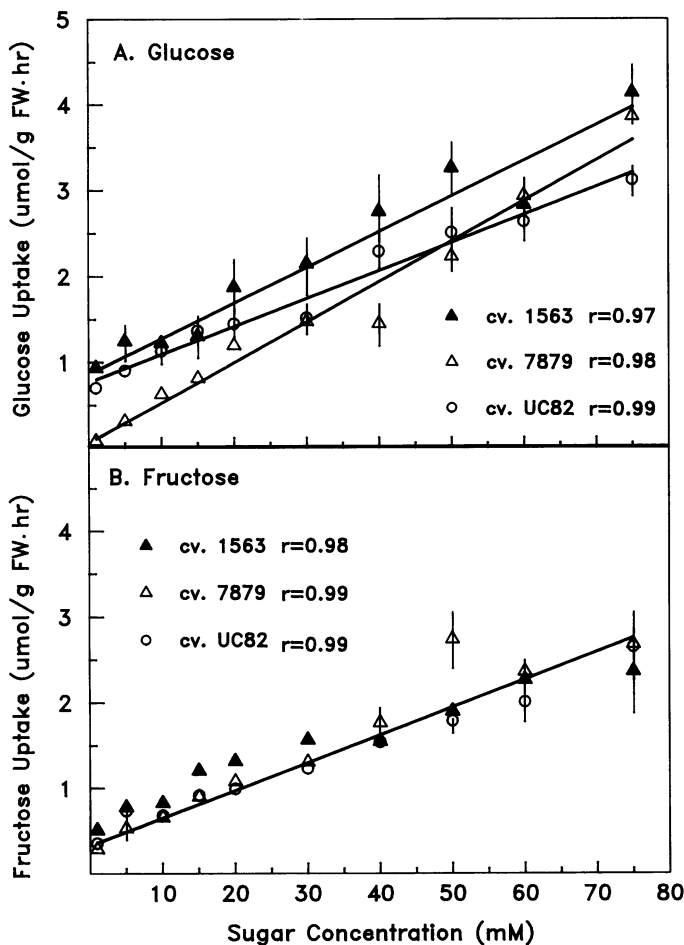


FIG. 4. Concentration dependence of glucose (A) and fructose (B) uptake in pericarp discs from fruit of cv 1563 (▲), cv 7879 (△), and cv UC82 (○). The data were analyzed by linear regression with correlation coefficient (r) for each line being 0.97 or greater. For clarity, only a single regression line is shown in panel B. Each point represents the mean \pm SE of three replicates.

essentially identical to the uptake rate of sucrose (Fig. 7). This result suggests that hydrolysis of sucrose by extracellular invertase is not required for uptake by tomato pericarp cells. Similar results have recently been reported for sucrose uptake in maize endosperm (8).

Treatment of tomato pericarp discs with 10 μM CCCP or 10

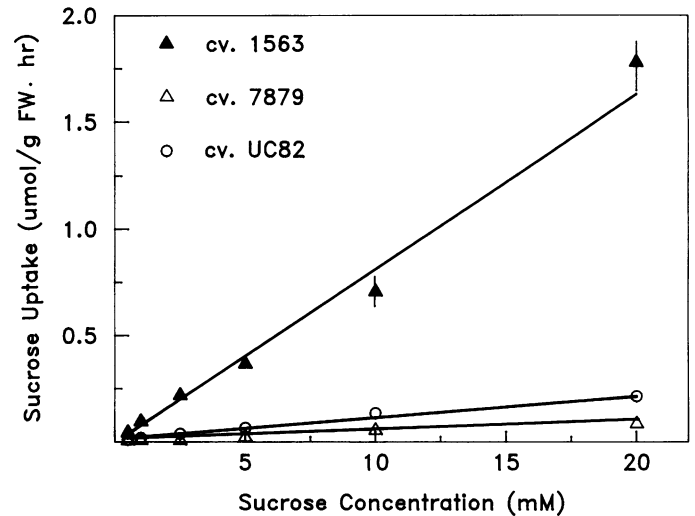


FIG. 5. Concentration dependence of sucrose uptake in pericarp discs of cv 1563 (▲), cv 7879 (△), and cv UC82 (○). Each point represents the mean \pm SE of five replicates.

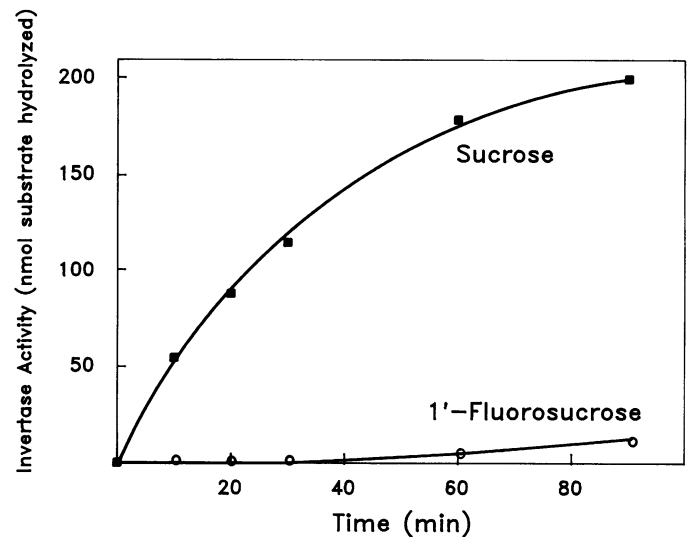


FIG. 6. Hydrolysis of sucrose or 1'-fluorosucrose by tomato invertase. The reaction was carried out in the presence of 10 mM substrate and 38 μg of partially purified tomato fruit invertase. Each point represents a single determination.

μM PCMBs had no effect on either glucose, fructose, or sucrose uptake when measured in short-term (30 min) experiments (Table II). This result suggests that the transport of sugars across the plasma membrane is not energy-dependent. Similar results have recently been reported for uptake of sugars in developing maize endosperm (9).

In long-term (3 h) experiments, however, accumulation of glucose in tomato pericarp tissue was inhibited by 10 μM CCCP (Fig. 8). 3-O-MG, a nonmetabolized glucose analog, was taken up at somewhat lower rates than glucose and uptake of 3-O-MG was not inhibited by CCCP (Fig. 8). Together, these results suggest that accumulation of sugars in tomato pericarp is not dependent on energy-dependent transport processes at the plasma membrane but in the long-term may be dependent on plasma membrane and/or possibly on energy-dependent transport processes localized in the tonoplast.

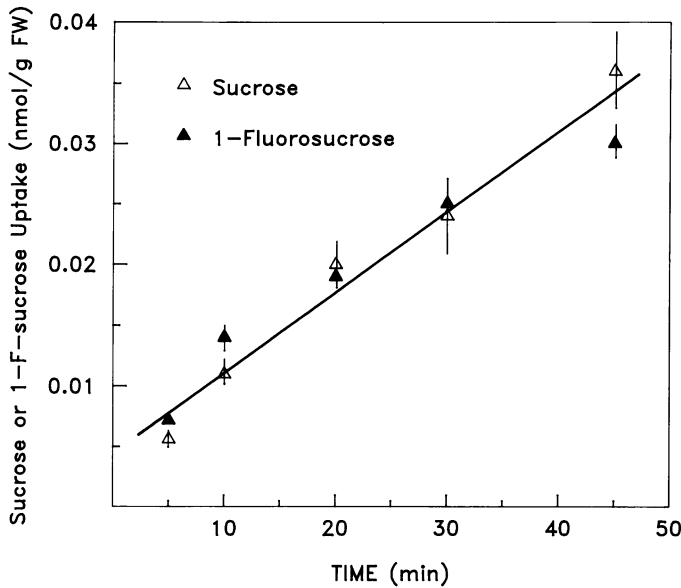


FIG. 7. Time course of uptake of sucrose and 1-fluorosucrose in pericarp discs of cv 7879. Each sugar was present at a concentration of 10 μ M. Values represent the mean \pm SE of three replicates.

CONCLUSIONS

The results presented suggest at least a partially apoplastic route of sucrose unloading in tomato fruit pericarp. The high ratios of apoplastic hexoses to sucrose and the appearance of an asymmetrically labeled apoplastic hexose pool following leaf labeling with [3 H]-[fructosyl]-sucrose further support the existence of extracellular invertase activity. It has been previously suggested that extracellular invertase plays a key role in carbon import in tomato fruit by maintaining a steep downhill sucrose concentration gradient between the phloem and the extracellular unloading site (5, 11).

In spite of the apparent presence of extracellular invertase, the results presented here indicate that sucrose may be taken up directly by pericarp cells. Thus, it appears that hexoses are not preferentially taken up and extracellular hydrolysis of sucrose does not appear to be required for sucrose uptake and utilization by pericarp cells. This result is consistent with the finding of Robinson *et al.* (19) that sucrose synthase activity is high early in tomato fruit development, a time when the rate of sucrose import is high (22). Sucrose synthase may provide the means for utilizing sucrose that escapes extracellular hydrolysis and is taken up directly by pericarp cells.

Nonsaturating uptake kinetics and lack of CCCP or PCMBS inhibition of glucose, fructose, or sucrose uptake suggest that sugar transport across the tomato pericarp plasma membrane is a passive process. A similar conclusion was made for sugar uptake in developing maize endosperm (9). Maintenance of a favorable gradient for sugar transport across the plasma membrane would then require further compartmentation either as starch, as occurs in early fruit development (19), or as soluble hexose in the vacuole. We have characterized components of the metabolic pathway contributing to transient starch accumulation (19) but know very little concerning the mechanism of sugar uptake and storage in the vacuole. Because our present results implicate vacuolar sugar storage as an important component of sugar accumulation in tomato fruit, mechanisms of sugar transport across the tonoplast need to be determined.

Table II. Effect of 10 μ M CCCP and 10 μ M PCMBS on Sugar Uptake in Tomato Pericarp Discs

Uptake was measured at a sugar concentration of 1 mM in pericarp discs at 20 DAA in cv 1563. Inhibitors were present at the indicated concentration 30 min prior to and during the 30 min uptake period. Each value is the mean and (standard error) of 3 replicates.

Inhibitor	Sugar Uptake		
	Glucose	Fructose	Sucrose
	<i>nmol/g FW</i>		
Control	27.1 (2.2)	23.6 (2.1)	16.9 (1.4)
PCMBS (10 μ M)	25.5 (2.1)	25.3 (2.8)	17.9 (2.4)
CCCP (10 μ M)	26.0 (2.2)	18.0 (4.3)	20.6 (1.2)
LSD	6.8	9.8	5.5

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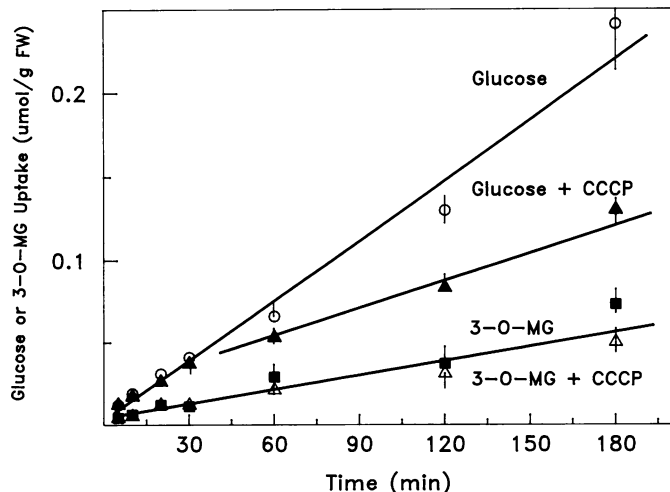


FIG. 8. Time course of uptake of glucose and 3-O-MG in the absence and presence of 10 μ M CCCP. Each point represents the mean \pm SE of three replicates.

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